IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: 27048U

In re Application of: Moshe Baru et al.

Serial No.: 10/553,357 Art Unit: 1654

Date Filed: July 13, 2006 Examiner: Ha, Julie

For: PHARMACEUTICAL COMPOSITION COMPRISING PROTEINS AND/OR POLYPEPTIDES AND COLLOIDAL PARTICLES

DECLARATION UNDER 37 CFR §1.132

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Sir:

I, Moshe Baru, an Israeli citizen residing at Hahadarim Pinat Tarpat St., Pardes Hana, Israel, hereby declare and state as follows:

1. I am the General Manager and Chief Scientist, Omri Laboratories Ltd, Nes-Ziona, Israel, a wholly-owned subsidiary of Opperbas Holding B.V., the assignee of record in the above-identified application, and my educational and professional experience is presented in the curriculum vitae attached hereto (Annex A).

2. I am a co-inventor and am familiar with the contents of U.S. Application No. 10/553,357 (hereinafter: *the application*). The application describes a pharmaceutical composition for parenteral administration comprising a therapeutically

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effective amount of a protein or polypeptide and colloidal particles as defined in the claims (hereinafter: *the invention*).

3. Although the application describes results regarding the treatment of hemophilia, these results are representative of other diseases which may be treated by the proteins and polypeptides used in the composition of the invention. In the attached Annex B, I describe experimental results using a composition comprising G-CSF prepared according to the invention for the treatment of neutropenia and for mobilization of stem cells into peripheral blood. These indications differ significantly from hemophilia.

4. It may be seen from the results of the experiments that the composition of the invention may be used to enhance the therapeutic effect of the proteins and polypeptides defined in the application.

5. Furthermore, the above results provide a reasonable basis for the assumption that a composition comprising G-CSF prepared according to the invention may be used to treat other diseases for which G-CSF is known to be effective, including multiple sclerosis (as indicated in Tompkins, SM and Miller, SD, Nature Medicine, 2002, 8:451-453 [cited by the Examiner]).

6. The undersigned declares further that all statements made herein of my own knowledge are true and that all statements

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made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

23.12.07
Date

Moshe Baru
Dr. Moshe Baru

Annex A

Personal details

Name: Moshe Baru

Address: Hadarim Street, Pardes-Hanna 37012, Israel

Date of Birth: 22/11/1960

Education

1986	B.Sc. (with honors), Hebrew University, Jerusalem, Israel
1992	PhD (direct PhD program), The Technion, Israel Institute of Technology, Haifa, Israel.

Positions and Employment

1992 - 1995	Senior scientist, Octa Medical Research Institute
1995 - 1997	Group leader and head of projects, Omri Laboratories Ltd.
1997 - 2001	Head of research and development, Omri Laboratories Ltd.
2001 -	General Manager and Chief Scientist, Omri Laboratories Ltd, Weizmann Science Park, Nes-Ziona, Israel.

Honors

1984 Dean's List for highest performing BSc students, Hebrew University, Jerusalem

1985 Dean's List for highest performing BSc students, Hebrew University, Jerusalem

1986 Dean's List for highest performing BSc students, Hebrew University, Jerusalem

Publications .

1. Baru, M. and Manor, H. Induction of polyomavirus DNA replication by cyclic AMP and a tumor promoter. Intervirology (1988) 29: 328-333.

2. Baru, M., Schlissel, M. and Manor, H. The yeast GAL4 protein transactivates the polyomavirus origin of DNA replication in mouse cells. Journal of virology (1991) 65:3496-3503.

3. Baru, M., Schlissel, M. and Manor, H. Induction of polyoma DNA replication by carcinogens in polyoma transformed rat cells - evidence that the viral enhancer is not the primary target in the induction pathway. Journal of Virology (1992) 66: 1261-1266.

4. Baru, M., Axelrod, H .J. and Nur, I. Liposome-encapsulated DNA mediated gene transfer and synthesis of human factor IX in mice. Gene (1995) 161: 143-150.

5. Baru, M., Sha'anaani, J. and Nur, I. Retroviral-mediated in-vivo gene transfer into muscle cells and synthesis of human factor IX in mice. Intervirology (1995) 38:356-360.

6. Baru, M., Nahum, O., Jaaro, H., Sha'anani, J. and Nur, I. Lysosome-disrupting peptide increases the efficiency of in vivo gene transfer by liposomes-encapsulated DNA. Journal of Drug Targeting (1998) 6:191-199.

7. Baru, M. Gene therapy of hemophilia A (1998) Chemistry 42: 20-23 (in Hebrew).

8. Otsuka, M., Baru, M., Nur, I. Gianello, P. In vivo gene transfer into rat and pig liver by large multilamellar liposome-encapsulated DNA. Journal of Drug Targeting (2000) 4: 267-279

9. Baru M, Carmel-Goren L, Barenholz Y, Dayan I, Ostropolets S, Slepoy I et al. Factor VIII efficient and specific non-covalent binding to PEGylated liposomes enables prolongation of it's circulation time and haemostatic activity. *Thromb Haemost* 2005; **93**: 1061-8.

10. Yatuv R, Dayan I, Baru M. A modified chromogenic assay for the measurement of very low levels of factor VIII activity (FVIII:C). Haemophilia. 2006; **12**:253-7.

Patents

1. Barenholz, Y., Bar, L. Nur, I. and Baru, M. A method of preparation of vesicles loaded with biological structures, biopolymers and/or oligomers. US patent 6,066,331 (2000).

2. Baru, M. and Nur, I. Nucleic acid delivery vehicle. US patent 6,207,456 (2001).

3. Barenholz, Y., Bar, L. Nur, I. and Baru, M. A method for high loading of vesicles with biopolymeric substance. US patent 6,156,337 (2001).

4. Baru, M. Bar, L. and Nur, I. Pharmaceutical composition for the treatment of blood coagulation disorders. US patent 6,593,294 (2003).

5. Baru, M. Liposome-mediated DNA administration. European patent 1361863 (2007).

ANNEX B

1. Treatment of Neutropenia

Materials and Methods

Reagents
Cyclophosphamide (Endoxan - Baxter, Deerfield, IL, USA)
G-CSF (Neupogen - Roche, Basal, Switzerland) - 48MU/0.5ml syringe (960μg/ml)

Animals
Male Spargue-Dawley (SD) rats (6-8 weeks old, ~200g) and guinea pigs (6-8 weeks old, ~400g) were used for animal studies. Animal care was in accordance with the U.S. National Institutes of Health guidelines. Research protocols were approved by the Institutional and the National Animal Care Committees.

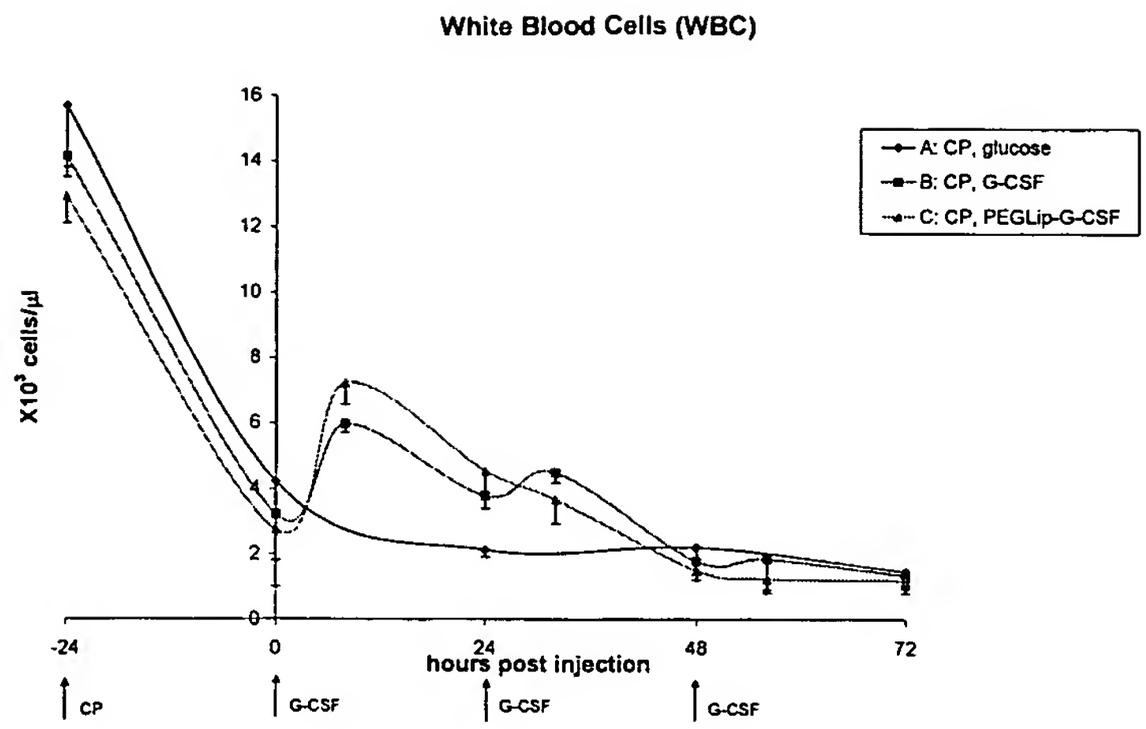
Formulation of G-CSF and liposomes
G-CSF (960μg/ml) was diluted with PEGylated liposomes (PEGLip) solution according to the invention or 5% glucose to a final concentration of 20μg/ml and incubated for 20 min at room temperature.

Results

Rat experiments
All animals received a single intraperitoneal (i.p) dose of 100mg/kg cyclophosphamide (CP) at day 0 to induce neutropenia. On days 1, 2, and 3 rats received a single daily subcutaneous (s.c) injection of vehicle (5% glucose), 30μg/kg of free G-CSF, or 30μg/kg of PEGylated liposome-formulated G-CSF (PEGLip-G-CSF). All rats were weighed daily. Rats treated with G-CSF were bled prior to CP injection, prior to the first G-CSF injection, and at 8, 24, 32, 48, 56, and 72 hours following the first G-CSF injection. Control rats were bled prior to CP injection, prior to the first injection of vehicle, and at 24, 48, and 72 hours following the first injection of vehicle. White blood cell counts and differential counts of neutrophils, eosinophils, basophils, monocytes and lymphocytes were determined for each blood sample. The results are presented in Figure 1 and Table 1.

Figure 1

A.



B.

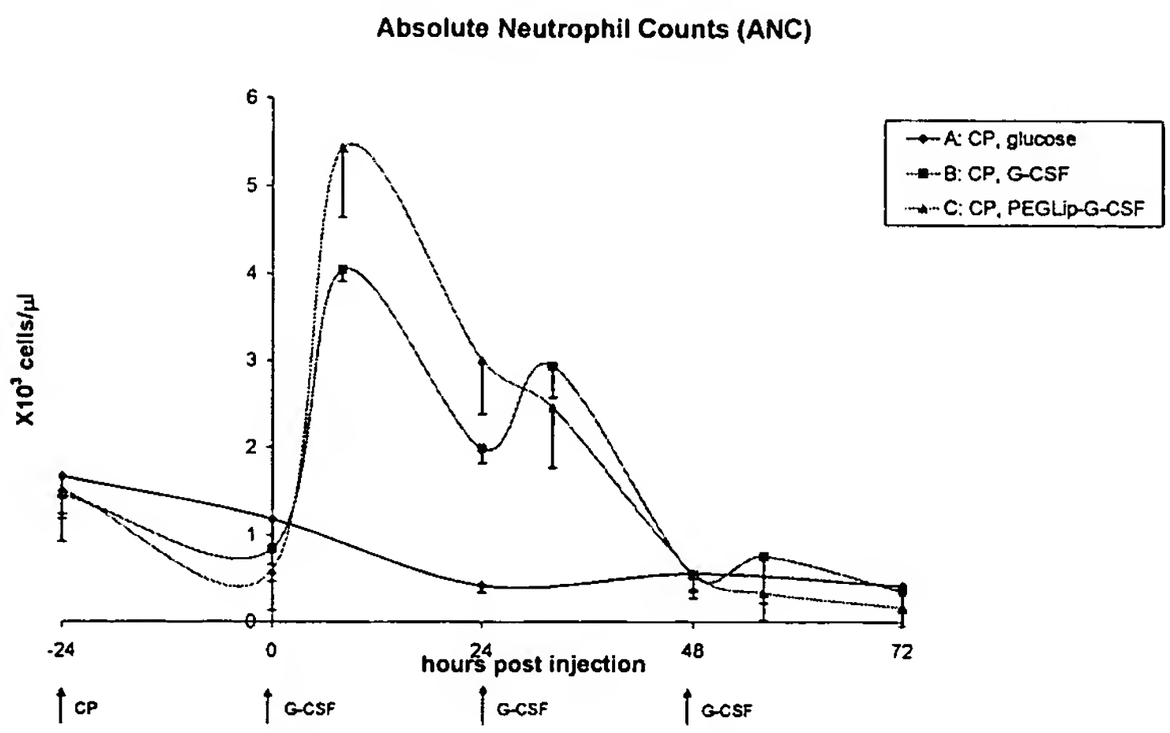


Figure 1: White blood cell counts (A) and absolute neutrophil counts (B) following injection of glucose, free G-CSF, and PEGLip-formulated G-CSF into neutropenic SD rats.

Table 1

	AUC WBC	PEGLip-G-CSF/ G-CSF	AUC ANC	PEGLip-G-CSF/ G-CSF
A: CP, glucose	0		0.07 ± 0.14	
B: CP, G-CSF	5.48 ± 3.14		6.78 ± 1.51	
C: CP, PEGLip-G-CSF	8.58 ± 5.31	**156.60%**	9.49 ± 1.38	**140%**

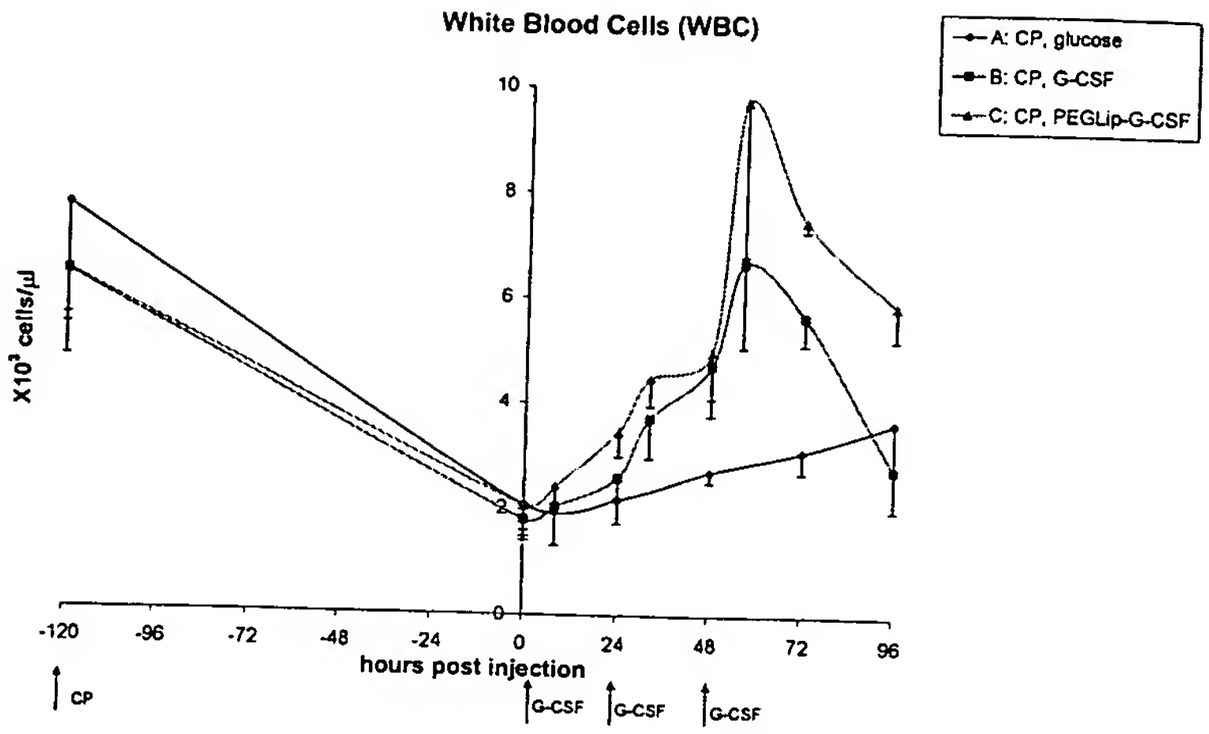
Table 1: Area under the curve (AUC) of white blood cells (WBC) and absolute neutrophil counts (ANC) in neutropenic SD rats.

Guinea pig experiments

All animals received a single i.p dose of 100mg/kg CP at day 0 to induce neutropenia. On days 5, 6, and 7 guinea pigs received a single daily (s.c) injection of vehicle (5% glucose), 30μg/kg of free G-CSF, or 30μg/kg of PEGLip-G-CSF. All guinea pigs were weighed daily. Guinea pigs treated with G-CSF were bled prior to CP injection, prior to the first G-CSF injection, and at 8, 24, 32, 48, 56, 72, and 96 hours following the first G-CSF injection. Control guinea pigs were bled prior to CP injection, prior to first injection of vehicle, and at 24, 48, 72 and 96 hours following the first injection of vehicle. White blood cell counts and differential counts of neutrophils, eosinophils, basophils, monocytes and lymphocytes were determined for each blood sample. The results of guinea pig experiments are presented in Figure 2 and Table 2.

Figure 2

A.



B.

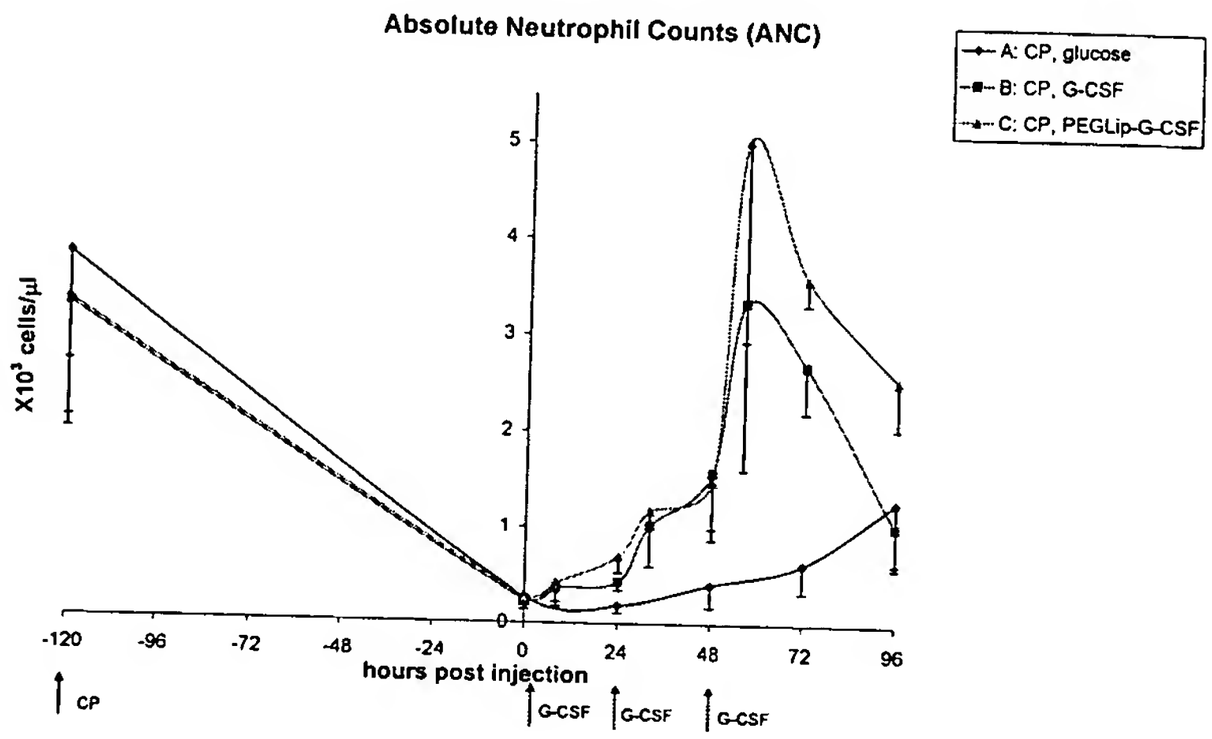


Figure 2: White blood cell counts (A) and absolute neutrophil counts (B) following injection of glucose, free G-CSF and PEGLip-formulated G-CSF into neutropenic guinea pigs.

Table 2

	AUC WBC	PEGLip-G-CSF/ G-CSF	AUC ANC	PEGLip-G-CSF/ G-CSF
A: CP, glucose	3.46 ± 2.27		1.58 ± 1.31	
B: CP, G-CSF	15.60 ± 3.33		8.75 ± 2.98	
C: CP, PEGLip-G-CSF	24.00 ± 6.19	**153.80%**	12.99 ± 4.59	**149%**

Table 2: Area under the curve (AUC) of white blood cells (WBC) and absolute neutrophil counts (ANC) in neutropenic guinea pigs.

Conclusions

- PEGLip-G-CSF is more effective than free G-CSF in enhancing white blood cells and neutrophils counts in both neutropenic rats and guinea pigs.
- Injection of PEGLip-G-CSF resulted in an increase of ~50% in white blood count AUC and absolute neutrophil count AUC versus that of free G-CSF.

2. Mobilization of stem cells into peripheral blood

Material and methods

Neupogen (96μg/ml) was mixed with 5% glucose or 9% PEGLip solutions to a final G-CSF concentration of 75μg/ml (final liposome concentration in the mix was ~8.3%). Mixtures were incubated for 20 min at room temp, rolling.

Balb/C male mice were injected IV with 5% glucose solution or with 300μg/kg of each of G-CSF formulations (Free or PEGLip). Mice were injected daily for 5 days, and were bled 3h after the last injection (99h) from the retro-orbital sinus into EDTA tubes.

Mice blood of each group was pooled, and blood cells were washed with PBS/ 0.5% BSA. Washed packed cells were FcR blocked with mouse IgG to reduce non-specific antibody binding, and then incubated with FITC labeled antibodies against lineage markers (CD3, CD45R, CD11b, Gr-1) and PE labeled anti stem cell antigen 1 (sca-1) antibody. Cells were analyzed by flow cytometry and the number of stem cells (Lineage negative and sca-1 positive cells) in peripheral blood was determined.

Results

The results are presented in Table 3.

Table 3

Treatment	Sca-1 positive cells/μl blood
Glucose (control)	12
G-CSF	17
PEGLip-G-CSF	44

Table 3: Mobilization of stem cells into peripheral blood by G-CSF and PEGLip-G-CSF

Conclusion

PEGLip-G-CSF is more effective than free G-CSF in enhancing mobilization of stem cells into the peripheral blood.